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Evaluation of Hemoglobinopathies in Anemic Patient Using Red Cell Indices from a Five-Part Differential Cell Counter and HPLC Estimation of Abnormal Hemoglobin

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INTRODUCTION

Thalassemia and hemoglobinopathies are a group of autosomal-recessive inherited human disorders and are prevalent in many parts of the world. Heterozygote screening and genetic counselling are essential for the prevention and control of severe thalassemia diseases. Conventional methods, including erythrocyte indices and morphology, Hb electrophoresis, quantitation of Hb A2, Hb E, and Hb F, and detection of erythrocytes containing Hb H inclusion bodies, provide an accurate diagnosis.[1] HPLC **(High performance liquid chromatography)** is a sensitive and precise method for detecting thalassemia and abnormal HbS. It has become the preferred method for thalassemia screening because of its speed and reliability. An automatic HPLC system (Bio-Rad) has been developed primarily for the detection of β-thalassemia disorders such as β-thalassemia carriers, Hb S and Hb C. But information is limited about using such a system to study the complicated α- thalassemia and β-thalassemia syndromes in Southeast Asia. [1] Regarding significance of RBC indices, [2] concluded that RBC indices can be utilized for screening of thalassemia and other hemoglobinopathies and are to be confirmed by the HPLC method. They compared RBC indices of normal subjects with different hemoglobinopathies, and they found most of the RBC indices having a significant difference [2].

Thalassemia has burden on the healthcare systems of many countries.[3]

A screening project based on 56,814 college students and pregnant women recruited in the states of Maharashtra, Gujarat, Punjab, Karnataka, West Bengal and Assam indicated a carrier rate of 2.78% (Mohanty et al. 2008). Unfortunately, there are no adequately representative data sets to confirm or deny these approximations, and with 50,000–60,000 strictly endogamous communities in India (Gadgil et al. 1998), it is dubious whether any average disease prevalence estimate could realistically be applied to each and every community and sub-population.[4].

INCLUSION & EXCLUSION CRITERIA:

I. For Screening for anemia.

a. Participants were patients with clinical features of anemia

b. Should not be having nutritional deficiency features

II. For HPLC study.

a. Only participants who were having CBC findings favouring Hemoglobinopathies.

b. Participants will also be known cases of hemoglobinopathies based on clinical findings and are on blood transfusion and willing to get their HPLC study.

c. First and second-degree relatives of known cases of hemoglobinopathies.

III. Proposed intervention: After taking consent and assent, the intended intervention was the withholding of regular transfusion to obtain participants for actual type of haemoglobin. A regular CBC was carried out at the interval of 7 days during withholding of regular transfusion and clinical condition in OPD was assessed regularly.

IV. Vulnerable subjects: For paediatric patients CBC Sample was collected under the supervision of senior most technician.The procedure which was most vulnerable was withholding the procedure for obtaining the accurate abnormal haemoglobin level and was carried out under supervision of paediatrician.

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OBJECTIVES

Primary Objective:

1. Evaluation of all anaemia patient by 5-part differential cell counter machine.
2. Correlation of various CBC (complete blood count) parameters/red cell indices with severity and type of anaemia.
3. Preliminary identification of the anemia patient based on CBC parameters which favorsC hemoglobinopathies.
4. Subjecting the preliminary diagnosed hemoglobinopathy samples to study abnormal heamoglobin by HPLC.

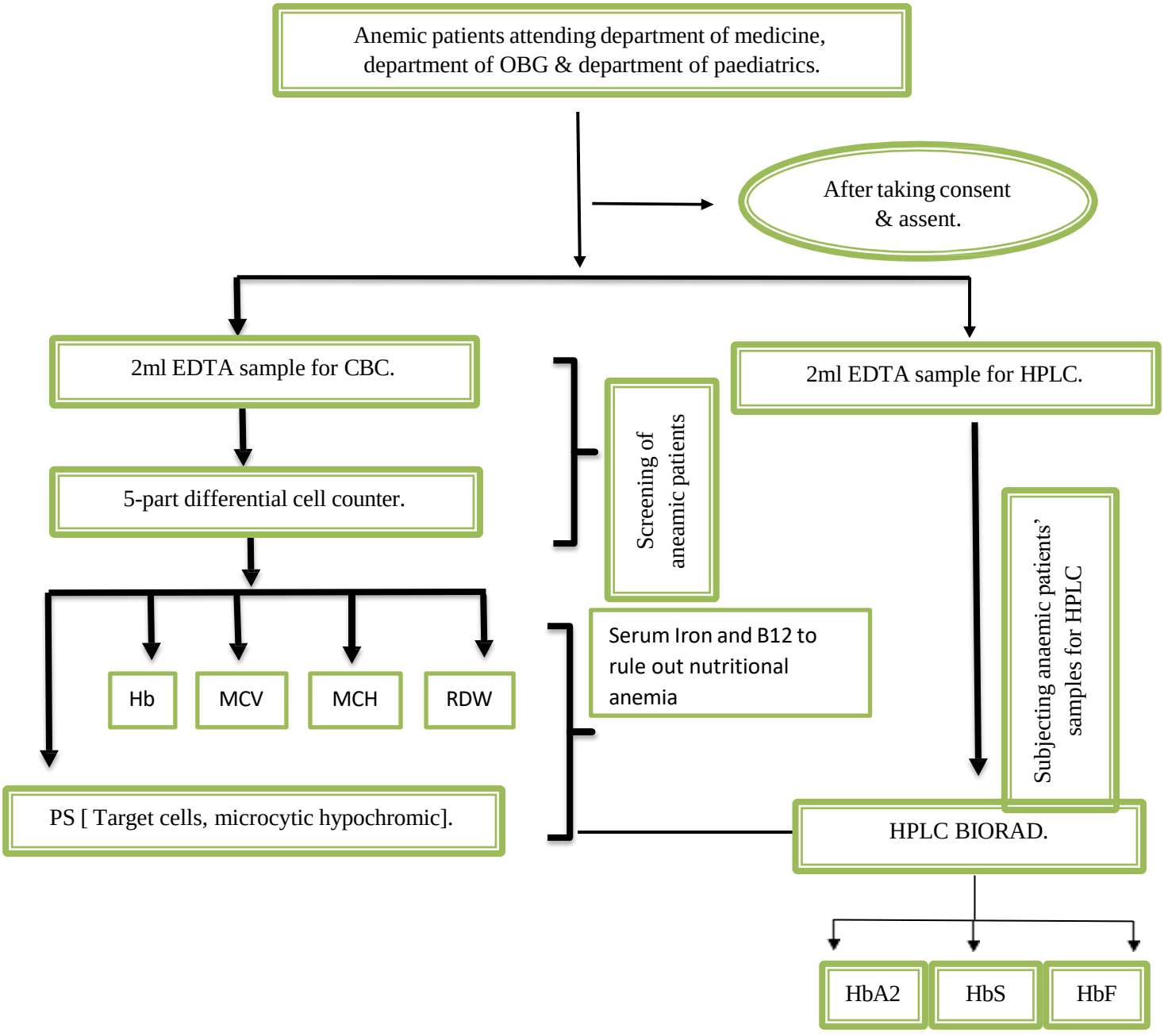
4.2 Secondary Objective:

1. Correlating the HPLC findings with various CBC parameters.

Participant recruitment procedures: All the anaemic patient based on the clinical findings favouring anaemia other than nutritional anaemia and anaemia due to blood loss were subjected for initial screening by 5-part differential cell counter and based on the red cell indices favouring hemoglobinopathies, this sample was subjected to HPLC by BIORAD detail.

METHODOLOGY

Type of study design: Retrospective Observational Study.



Study population: The study was carried out in a tertiary care hospital which is situated among the population of 11 lakh in Kalaburagi, a district of north Karnataka, India.

Types of data collection:

- Questionnaires based on general physical examination.
- 4ml EDTA sample, 2ml in each vacutainer, 1 EDTA container was utilised for red cell indices and 5-part cell counter analysis while the other EDTA container was used for HPLC.
- When serum iron and vitamin B12 levels were within normal range, sample was processed for HPLC. However, if they were decreased, patients were given treatment for the same and if there was no response, HPLC was considered at a later stage.

Sample size: 349.

Instrument used:

For CBC/Red cell Indices: The instrument used is Erba's 5-part differential cell counter (H560) and it's specifications are:

Electrical impedence method for RBC enumeration, 15 µl sample volume, throughput 60 samples/ hour,Bidirectional interfacing, QR Code scanner facilitating direct input of QC values.

Instrument used for HPLC: BIO-RAD D-10 Hemoglobin testing system

PRINCIPLES OF OPERATION OF HPLC BIORAD

The D-10 Haemoglobin Testing System uses the principles of high-performance liquid chromatography (HPLC). A dual-piston, low pulsation HPLC pump and a proportioning valve deliver the buffer solution to the analytical cartridge and detector. Whole blood samples undergo an automatic two-step dilution process and are then introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microbial adapter in the sample rack, and the automatic dilution step is omitted. Pre-diluted samples are aspirated directly and introduced into the analytical flow path. Between sample injections, the sample probe is rinsed with Wash/Diluent Solution to minimize sample carryover. A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge, where the haemoglobins are separated based upon their ionic interactions with the cartridge material. The separated haemoglobins then pass through the photometer flow cell where changes in the absorbance are measured at 415 nm. The software performs a reduction of raw data collected from each analysis that may include use of a calibration factor. A sample report and chromatogram are generated for each sample.

Serum Iron and B12 was performed by Department of biochemistry by AU 480 Beckman coulter chemistry analyzer machine.

BIO-RAD D-10 Hemoglobin testing system

QUALITY CONTROL

For H560 CBC 5-part cell counter: The level of control in our laboratory is three set with low, normal and high levels, referred by ISO 15189:2013[(5)] Frequency of this control is after every 300 samples.

For HPLC: Primer and calibrators with every cartridge and two controls, low, and high were used as quality control**.**

MATERIALS AND METHOD

This study was a retrospective study carried out in the department of pathology in KBNTGH. A total of 349 patients were screened and were included in the study. Initially they were screened for anaemia and were subjected for HPLC to find out abnormal haemoglobin. This also included the patients who were not responding to treatment who had visited for the first time to KBNTGH, clinically suspected patients of hemoglobinopathies and the relatives of abnormal haemoglobin patients.

1. Privacy and confidentiality of the study participants:

In cloud with password protection.

2. Data Analysis:

a. Descriptive and summary measures like mean and standard deviation were used to estimate CBC findings and HPLC findings.
b. Prevalence of anemia was summarized using frequency and percentage.
c. Association between CBC and hemoglobinopathies was assessed using Chi-Square test.
d. Linear regression method was used to predict the HPLC among anemia cases.
e. Karl pearson's correlation method was used to measure the correlation between CBC and severity of anemia.
f. Data analysis was performed using IBM SPSS and data was managed with MS EXCEL.

Procedure for seeking and obtaining written informed consent with a sample of the

Patient /participant information sheet

Informed consent forms in English and local languages. Informed consent for storage of samples /assent; re-consent

RESULTS AND OBSERVATIONS

Table No.1: Age wise distribution anaemic patients

Age in years	Number of patients	Percentage
0—19	51	14.6
20—39	178	51.0
40—59	67	19.2
≥ 60	53	15.2
Mean ± SD	34.54 ± 19.13	

Study observes that, majority of patients 178 (51.0%) belonged to the age group of 20—39 years. Followed by 67 (19.2%) of patients who belonged to the age of 40—59 years, 53 (15.2%) of patients who belonged to the age of ≤ 60 years and 51 (14.6%) of patients who belonged to the group of 0-19 years. The minimum age of patient was 3 months and maximum age of patient was 94 years. The mean age of patients was 34.54 years

Simple bar diagram represents age wise distribution of patients

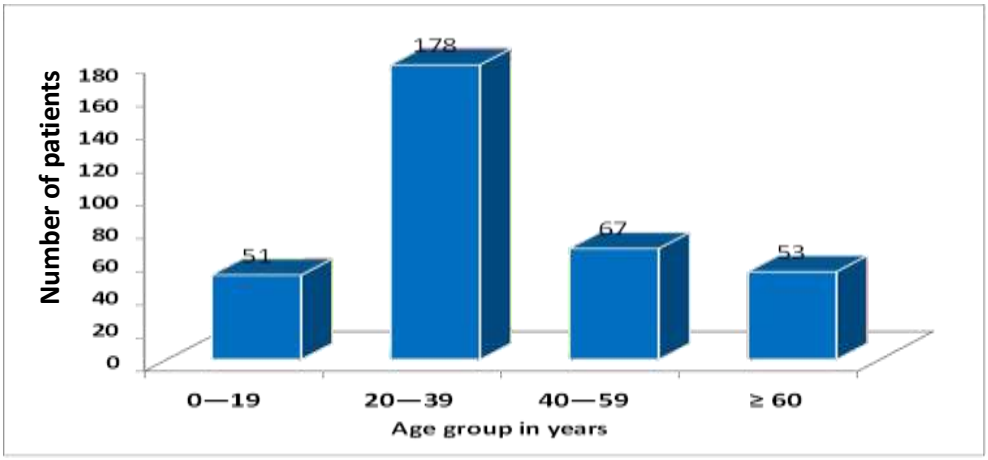
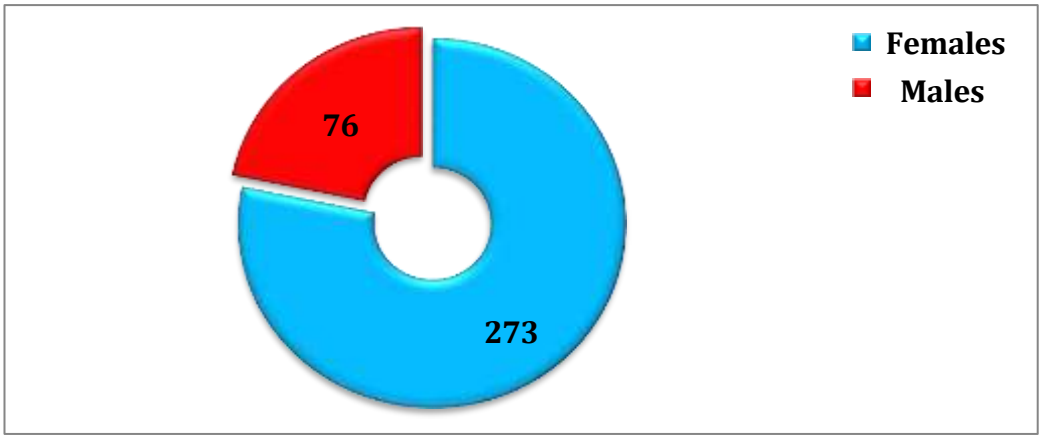


Table No.2: Gender wise distribution anaemic patients

Gender	Number of patients	Percentage
Females	273	78.2
Males	76	11.8

In the study female patients were predominant 273 (78.2) and male patients were 76 (11.8%)



Pie diagram presents gender wise distribution anaemic patients

Table No.3: Distribution anaemic patients according to departments

Departments	Number of patients	Percentage
ENT	1	0.3
MEDICINE	198	56.7
OBG	102	29.3
OPTHALMOLOGY	9	2.6
ORTHOPEDICS	5	1.4
PEDIATRICS	14	4.0
SURGERY	20	5.7
Total	349	100.0

Study observed that; majority of anaemic patients 198 (56.7%) belonged to medicine department, followed by 102 (29.3%) of patients who belonged to OBG department.

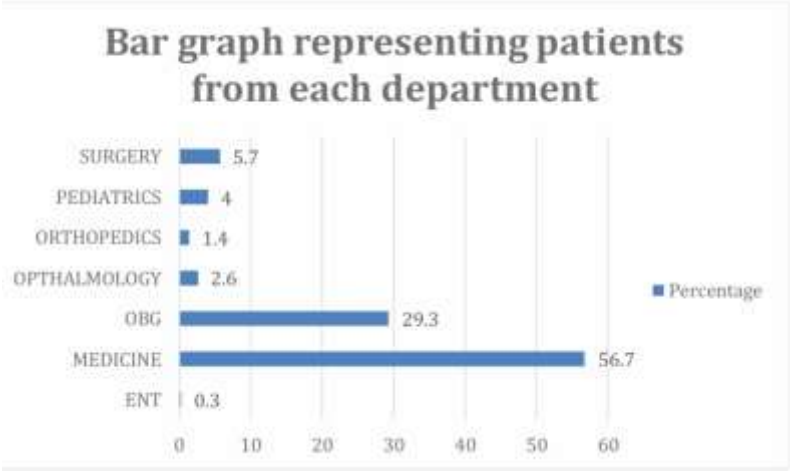


Table No.4: Distribution anaemic patients with CBC parameters

Variables		Number of patients	Percentage
Hb	Normal	0	0.0
	Abnormal	349	100.0
MCV	Normal	64	18.3
	Abnormal	285	81.7
RBC	Normal	62	13.3

	Abnormal	287	86.7
MCH	Normal	87	24.9
	Abnormal	262	75.1
RDW	Normal	206	59.1
	Abnormal	143	40.9

Hb% level seen all the 349 patients (100.0%) was abnormal, 285 (81.7%) patients were abnormal for MCV, 287 (86.7%) patients were abnormal for RBC, 262 (75.1%) patients were abnormal for MCH and 143 (40.9%) patients were abnormal for RDW.

Table No.5: HPLC wise distribution anaemic patients

HPLC		**Number of patients**	**Percentage**
HbA2	Done	63	18.1
	Not done	286	81.9
HbF	Done	63	18.1
	Not done	286	81.9
HbS	Done	63	18.1
	Not done	286	81.9

In the study out of 349 anaemic patients 63 (18.1%) patients' HPLC investigation was carried out.

Table No.6: Serum Iron and B12 investigations wise distribution anaemic patients

Serum Iron and B12		**Number of patients**	**HPLC DONE**	**Percentage**
Serum Iron	↓	189	4	54.3
	↓↓	56	0	16.0
	Normal	55	0	15.7
	Not done	49	0	14.0
Serum B12	↓	2	0	0.57
	↓↓	1	0	0.28
	Not done	346	0	99.1

Study observed that during serum iron analysis: Out of 300 (86.0%) patients whose serum iron analysis was done, 189 (54.2%) of patient's serum iron was decreased, 56 (16.0%) of patient's serum iron was significantly decreased, 55 (15.7%) of patient's serum iron was normal.

Serum B12 analysis; 346 (99.1%) of patients' serum B12 was not tested, 2 (0.6%) of patients serum B12 was decreased, 1 (0.3%) of patient's serum B12 was significantly decreased.

Table No.7: Comparison of HPLC of HbA2 with CBC variables

CBC	**CBC**	**HbA2**	Correlation coefficient	t-test, P-value
	Mean ± SD	**Mean ± SD**		
Hb	8.91 ± 1.47	3.15 ± 0.83	r = +0.232, P<0.05, S	P <0.00, HS
MCV	69.76 ± 11.56	3.15 ± 0.83	r = -0.245, P<0.05, S	P<0.001, HS
RBC	4.17 ± 0.84	3.15 ± 0.83	r = +0.272, P<0.01, HS	P<0.001, HS
MCH	23.38 ± 4.72	3.15 ± 0.83	r = -0.254, P<0.05, S	P<0.014, S
RDW	15.79 ± 2.78	3.15 ± 0.83	r = +0.204, P>0.05, NS	P<0.014, NS

There was statistically significant positive correlation between HbA2 with Hb and RBC (P<0.05). There was statistically significant negative correlation between HbA2 with MCV and MCH (P<0.05). There was statistically no significant correlation between HbA2 with RDW (P>0.05).

In our study the RDW was raised in the patients with iron deficiency anemia & few cases of vitamin B12 deficiency and these were not subjected to HPLC and patients responded to conventional treatment.

Table No.8: Comparison of HbF with CBC variables

CBC	CBC	HbF	Correlation coefficient	t-test, P-value
	Mean ± SD	Mean ± SD		
Hb	8.91 ± 1.47	0.81 ± 0.47	r = -0.352, P<0.01, HS	P <0.00, HS
MCV	69.76 ± 11.56	0.81 ± 0.47	r = -0.052, P>0.05, NS	P>0.312, NS
RBC	4.17 ± 0.84	0.81 ± 0.47	r = -0.321, P<0.01, HS	P<0.001, HS
MCH	23.38 ± 4.72	0.81 ± 0.47	r = +0.043, P>0.05, NS	P>0.314, NS
RDW	15.79 ± 2.78	0.81 ± 0.47	r = +0.031, P>0.05, NS	P>0.214, NS

There was statistically highly significant negative correlation between HbF with Hb and RBC (P<0.01). There was statistically no significant correlation between HBF with MCV, MCH and RDW (P>0.05)

Table No.9: Comparison of HPLC of HbS with CBC variables

CBC	CBC	HbS	Correlation coefficient	t-test, P-value
	Mean ± SD	Mean ± SD		
Hb	8.91 ± 1.47	1.88 ± 7.45	r = +0.252, P<0.05, S	P <0.00, HS
MCV	69.76 ± 11.56	1.88 ± 7.45	r = -0.139, P>0.05, NS	P>0.083, NS
RBC	4.17 ± 0.84	1.88 ± 7.45	r = +0.351, P<0.01, HS	P<0.001, HS
MCH	23.38 ± 4.72	1.88 ± 7.45	r = -0.105, P>0.05, NS	P>0.104, NS
RDW	15.79 ± 2.78	1.88 ± 7.45	r = +0.296, P<0.01, HS	P<0.001, HS

There was statistically significant positive correlation between HbS with Hb, RBC and RDW (P<0.05) and (P<0.01). There was statistically no significant correlation between HbS with MCV and MCH (P>0.05)

DISCUSSION

India is a religiously diverse country at having a practice of consanguineous marriages with all the religious beliefs hence the study was needed to create an awarenesses of increased probability of diseases with major health impact if not screened before marriages. Not necessarily the consanguineous marriages, random picking of the spouses also shown their offspring having a full-blown disease. HPLC showed a significant increase in the HbA2 levels and has got many advantages over other methods and is an excellent diagnostic tool for the identification of most of the clinically significant Hb variants specially to β-thalassemia trait owing to its quantitative power and automation. HPLC is sensitive, specific and reproducible and is less time consuming ideal for screening purpose. There was also the coexistence of increased HbA2 with iron deficiencies hence masking the characteristic CBC parameters of abnormal hemoglobin and were subjected to iron studies.

Hemoglobinopathies, including sickle cell disease (SCD) and thalassemia, are some of the most prevalent inherited disorders worldwide. In the study, the prevalence rates, alongside the impact on healthcare systems, are thoroughly evaluated. The research highlights the importance of understanding both the genetic distribution of these diseases and their healthcare burden, particularly in regions with high carrier rates. These findings align with prior studies emphasizing the need for robust early screening programs, especially in at-risk populations.

Early Screening and Outcomes

A significant portion of the study discusses early screening as a crucial intervention strategy. The findings suggest that early detection of hemoglobinopathies through neonatal and prenatal screenings can lead to timely interventions, which significantly improve patient outcomes. This mirrors previous literature which has shown that early diagnosis, especially in sickle cell disease, can reduce morbidity and mortality by allowing for early treatment and regular health monitoring [8] .

Management and Healthcare Strategies

The study further discusses the management strategies for hemoglobinopathies, stressing the importance of integrated healthcare approaches that include both genetic counseling and clinical management. The role of public health programs in raising awareness and providing resources for early detection and treatment is also emphasized. This is in line with the World Health Organization's recommendation on adopting national programs for controlling hemoglobinopathies, particularly in regions with high prevalence [9].

Genetic Counseling and Public Awareness

In terms of prevention, the study highlights the role of genetic counseling in reducing the incidence of hemoglobinopathies. Offering counseling services to high-risk couples can significantly reduce the number of affected births. Moreover, public health initiatives aimed at increasing awareness about the disease are critical in helping populations make informed reproductive choices. These

findings are supported by studies that show genetic counseling as an effective tool in managing genetic disorders [10].

LACUNAE IN THE STUDY

We had 6 patients who were thalassemia major and were on transfusion every 27 days. It was difficult to obtain the original Hb of the baby even after withholding the transfusion for 40 days under care. However, the strong clinical features favoured thalassemia major. In our next study we are planning for molecular characterisation of all the thalassemia trait and thalassemia major patients using non-hemopoietic DNA that would evade the necessity of withholding transfusion [7] .

Conflict Of Interest: None

Funding Agencies: None

CONCLUSION

This study allowed measurement of hemoglobinopathy prevalence in a specific geographical area, thereby reducing health-related burdens through effective counseling of the target population. The study will be useful for understanding the diagnosis and classification of anemia, both clinically and based on laboratory findings. It will aid in operating laboratory instruments and understanding protocols, SOPs, and quality control. Additionally, the study will help correlate various CBC parameters in anemia. The study underlines the need for comprehensive screening programs, public health interventions, and integrated management strategies to effectively address the burden of hemoglobinopathies. Early screening and awareness programs, coupled with effective healthcare policies, are vital to reducing both the prevalence and healthcare burden of these diseases.

REFERENCES

1) Fucharoen.S, Winichagoon.P, Wisedpanichkij. R, Sae-ngow.B, Sriphanich.R, Oncoung.W. Prenatal and postnatal diagnoses of thalassemia and hemoglobinopathies by HPLC. Clinical chemistry. 1998; 44:4-740.
2) Mia, Md Babu & Sadiya, Salma & Akhter, Nilufa & Sarwardi, Golam & Banu, Bilquis & Khan, Waqar. (2015). Role of RBC Indices in Screening of Thalassaemia and other Haemoglobinopathies in Children. 31. 99-106.
3) Amani Abu-Shaheen, Humariya Heena, Abdullah Nofal, Doaa A. Abdelmoety, Abdulrahman Almatary, Mohammed Alsheef, Isamme Al Fayyad, "Epidemiology of Thalassemia in Gulf Cooperation Council Countries: A Systematic Review", BioMed Research International, vol. 2020, Article ID 1509501, 15 pages, 2020. https://doi.org/10.1155/2020/1509501.
4) Sinha S, Black ML, Agarwal S, Colah R, Das R, Ryan K, Bellgard M, Bittles AH. Profiling βthalassaemia mutations in India at state and regional levels: implications for genetic education, screening and counselling programmes. Hugo J. 2009 Dec;3(1-4):51-62. DOI: 10.1007/ s11568-010- 9132-3. Epub 2010 Feb 10. PMID: 21119755; PMCID: PMC2882644.
5) Shivashankara A.R, Jailkhani R, Kini A. Hemoglobinopathies In Dharwad, North Karnataka: A Hospital-based Study. Journal of Clinical and Diagnostic Research [serial online] 2008 February [cited: 2008 February 4]; 2:593-599.Available from http://www.jcdr.net/back_issues.asp?issn=0973- 709x&year=2008&month= February.
6) GD, Kulkarni SS, Kadakol GS, Kulkarni BB, Kyamangoudar PH, Lakkakula BV, Thangaraj K, Shepur TA, Kulkarni ML, Gai PB. Molecular basis of β-thalassemia in Karnataka, India. Genet Test Mol Biomarkers. 2012 Feb;16(2):138-41. doi: 10.1089/gtmb.2011.0035. Epub 2011 Oct 6. PMID: 21978377.
7) Abd Rahim MR, Kho SL, Kuppusamy UR, Tan JA. Non-Invasive DNA Sampling for Molecular Analysis of Beta-Thalassemia: Amiable Alternative Sampling Methods with Accurate Results for Pediatric Patients. Clin Lab. 2015;61(9):1325-30. doi: 10.7754/clin.lab.2015.150218. PMID: 26554253.
8) Piel, F. B., Hay, S. I., Gupta, S., Weatherall, D. J., & Williams, T. N. (2017). Global burden of sickle cell anaemia in children under five, 2010–2050: Modelling based on demographics, excess mortality, and interventions. PLoS Medicine. 2017; 10(7).
9) World Health Organization (WHO).Sickle-cell anaemia: Report by the Secretariat.2006
10) Angastiniotis, M, Modell, B. Global epidemiology of hemoglobin disorders. Ann N Y Acad Sci. 1998;850(1): 251-269.